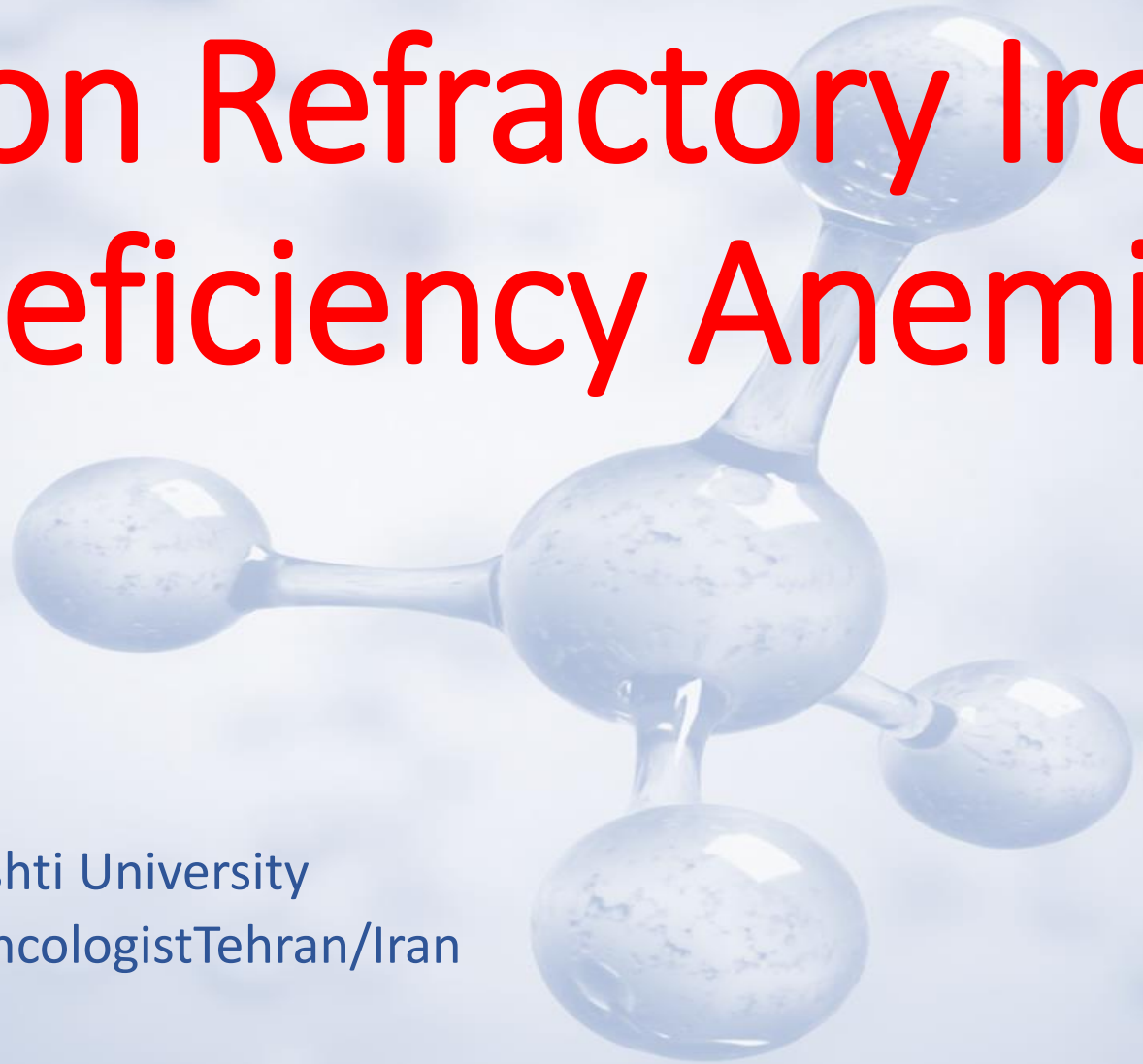
Iron Refractory Iron Deficiency Anemia



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Proteins involved in Iron Metabolism

- **Hepcidin (HAMP):** Is produced mainly by the hepatocytes and by inflammatory macrophages. Key negative regulator of intestinal iron absorption & macrophage iron release,** Hepcidin is the main regulator of iron homeostasis, it binds ferroportin, thus controlling the amount of iron released into the bloodstream. Mutations cause different forms of "HH".

- **Ferroportin (FPN1):** (cellular iron exporter): Ferroportin is a transmembrane protein that exports iron from the inside of a cell to the plasma. Ferroportin is regulated by hepcidin. Mutations of ferroportin cause a rare form of HH called "ferroportin disease".

- **Transferrin (Tf): (siderophilin):** plasma iron transport protein.

- **Transferrin receptor (TfR) and sTfR:** cellular and soluble receptor for transferrin

Proteins involved in Iron Metabolism

- **Iron regulatory protein 1 and 2 (IRP1 & IRP2):** Intracellular iron sensing protein
- **Divalent metal transporter 1:** (DMT1, Nramp2, DCT1: the duodenal iron transporter is located on the apical membrane of enterocytes.
- **Hephaestin (ferroxidase):** Cooperates with ferroportin for exporting iron to the plasma (transferrin).
- **Ceruloplasmin (Ferrooxidase):** Ceruloplasmin is the major copper-carrying protein in the blood and plays a role in iron metabolism. It exports iron from enterocytes, macrophages, hepatocytes and glial cells.

Proteins involved in Iron Metabolism

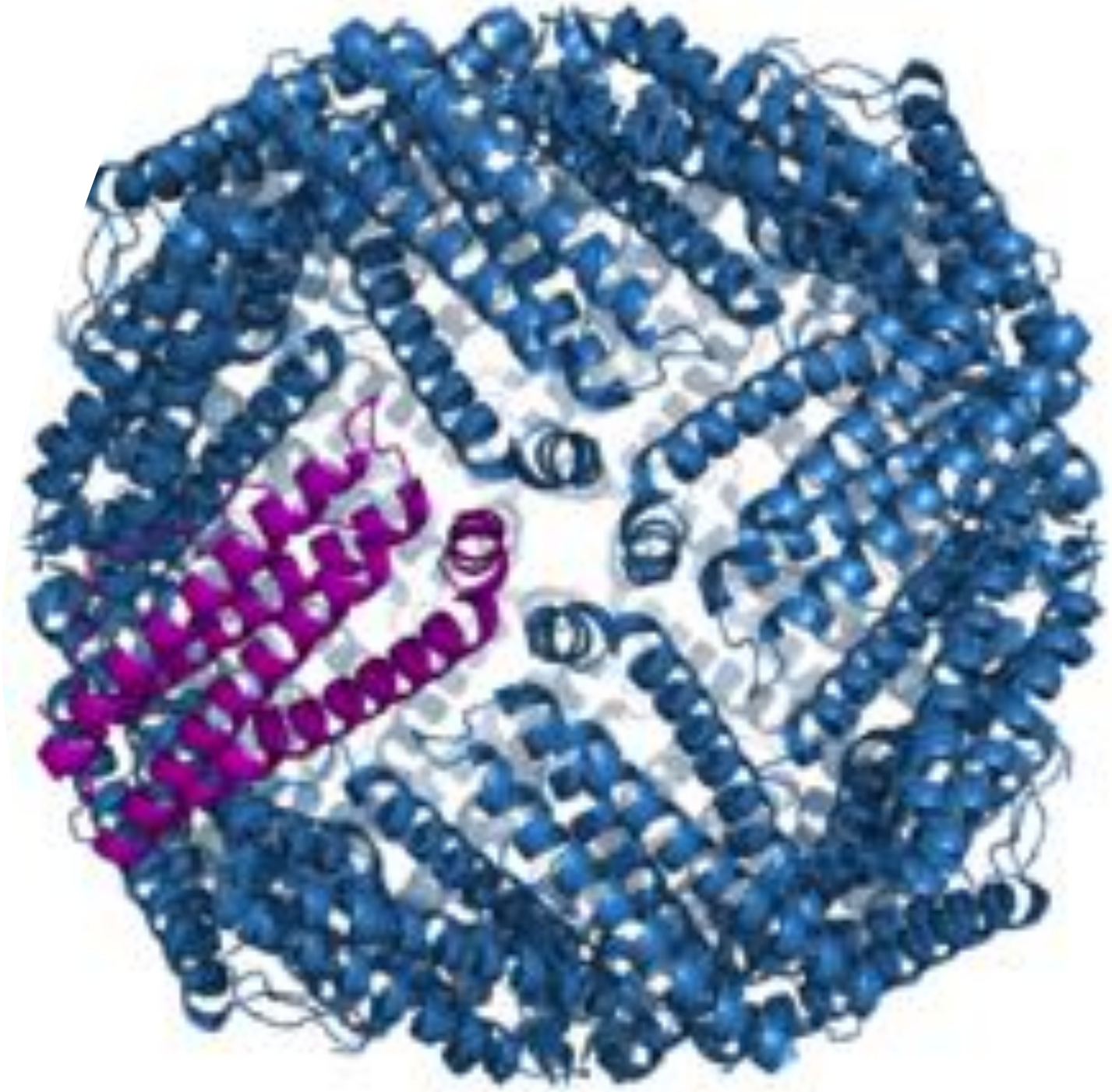
- **HFE:** In the liver, HFE activates hepcidin through a still unknown mechanisms. Inactivation of HFE in the liver results in a hemochromatosis-like phenotype
- **Hemojuvelin:** A positive hepcidin regulator, It functions as a BMP-coreceptor that positively modulates hepcidin. Inactivation of HJV in human causes juvenile hemochromatosis (type 2) and severe iron overload.
- **Bone morphogenic proteins (BMPs):** produced by endothelial cells stimulate hepcidin transcription by binding to BMP receptors
- **Matriptase 2/TMPRSS6:** (Liver hepcidin inhibitor). Matriptase-2 suppresses hepcidin transcription through degradation of hemojuvelin.
- **Erythroferrone:** protein hormone encoded in humans. Erythroferrone is produced by erythroblasts, inhibits the production of hepcidin in the liver, and so increases the amount of iron available for hemoglobin synthesis

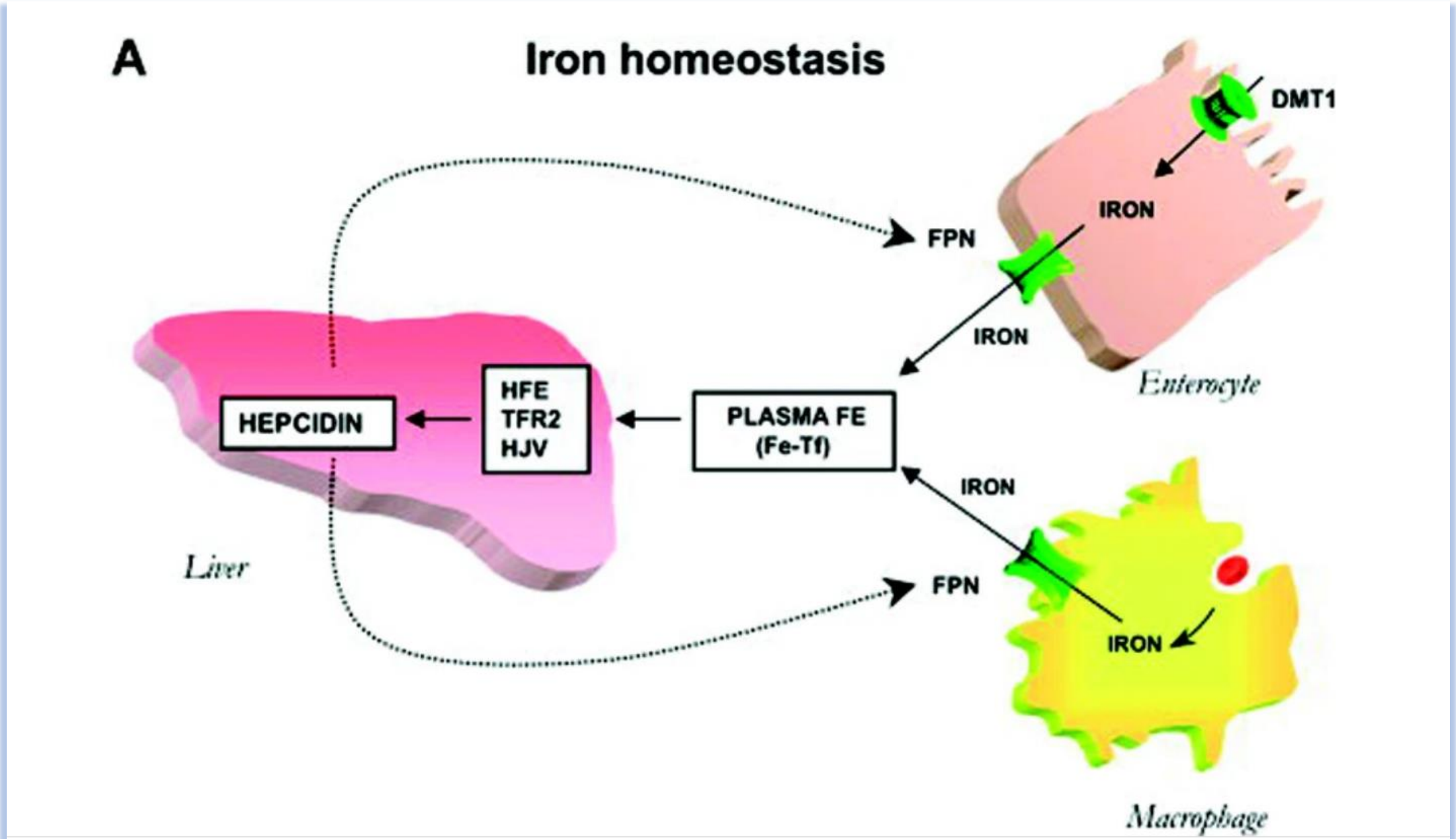
Ferritin

- **Ferritin** is a universal intracellular protein that stores iron and releases it in a controlled fashion.

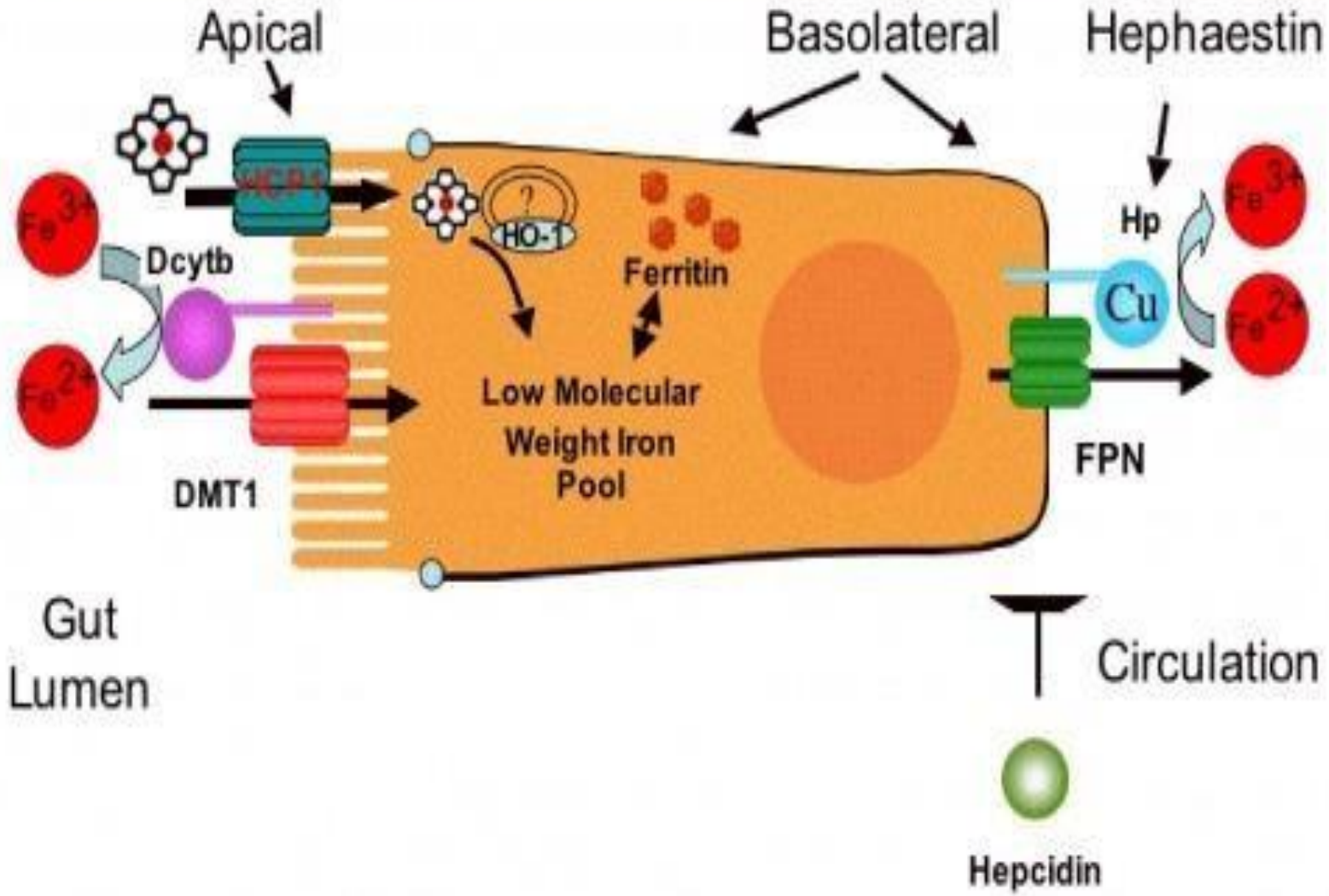
- The protein is produced by almost all living organisms, keeping iron in a soluble and non-toxic form. In humans, it acts as a buffer against iron deficiency and iron overload.

- Agregated ferritin transforms into toxic form of iron hemosiderin.




A
Iron homeostasis
DMT1
IRON
FPN
IRON
Enterocyte
HFE
TFR2
HJV
HEPCIDIN
PLASMA FE
(Fe-Tf)
IRON
Liver
FPN
IRON
Macrophage

Iron Transport through the Enterocyte



Hepcidin and Anemia: A Tight Relationship

- **Hepcidin, the master regulator of systemic iron homeostasis, tightly influences erythrocyte production.**
- **High hepcidin levels block intestinal iron absorption and macrophage iron recycling, causing iron restricted erythropoiesis and anemia.**
- **Low hepcidin levels increases BM iron supply for Hb synthesis and RBC production.**
- **Expanded erythropoiesis blocks hepcidin through the release of the erythroblast hormone of erythroferrone which is hepcidin inhibitor.**
- **Reduced erythropoiesis or limiting iron consumption increases transferrin saturation and stimulates hepcidin transcription.**

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Dysregulation of hepcidin synthesis is associated with anemia in three conditions

1) Iron refractory iron deficiency anemia (IRIDA) due to mutation in matriptase
2) Anemia of acute and chronic inflammatory disorders
3) Extremely rare hepcidin-producing adenomas

- Inappropriately high levels of hepcidin cause iron-deficient erythropoiesis in all these conditions.

- Patients with IRIDA or ACD do not respond to oral iron supplementation and show a delayed or partial response to intravenous iron.

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Hepcidin-related anemias

- Other hepcidin-related anemias are the “iron loading anemias” characterized by ineffective erythropoiesis and hepcidin suppression.

- This group of anemias includes thalassemia syndromes, congenital dyserythropoietic anemias, congenital sideroblastic anemias, and some forms of hemolytic anemias as pyruvate kinase deficiency.

- The paradigm is non-transfusion-dependent thalassemia, where the release of erythroferrone from the expanded pool of immature erythroid cells results in hepcidin suppression and secondary iron overload that in turn worsens ineffective erythropoiesis and anemia.

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The Iron-Erythropoiesis Connection

- Hepcidin regulation requires a crosstalk between liver endothelial sinusoidal cells that produce BMPs to activate the BMP-SMAD pathway and hepatocytes that produce and release hepcidin.

- Hepcidin levels are low in absolute iron deficiency and IDA. In these conditions, the iron stores are exhausted and the BMP-SMAD signaling is switched off at multiple levels.
- In IDA:

1) First, BMP6 expression is suppressed;

2) The activity of TMPRSS6, a protease that cleaves hemojuvelin is strongly increased

3)Histone deacetylase 3 (HDAC3) suppresses the hepcidin locus.

facilitates dietary and pharmacological iron absorption

When anemia is severe, the coexisting hypoxia stimulates erythropoiesis through increased synthesis and release of EPO.

suppression of hepcidin transcription occurs by:

- Erythroferrone (ERFE) produced by erythroblasts
- Release of molecules (e.g., PDGF-B) by other tissues
- Soluble components of transferrin receptors (TFR), sTFR1 and sTFR2.

supply enough iron for the needs of erythropoiesis

Anemias With Abnormal Hepcidin Levels

Anemia may be classified on the basis of hepcidin levels:

1) High hepcidin levels:

- Persistently high hepcidin levels; by blocking iron absorption, cause IDA.

2) Low hepcidin levels

- Ineffective erythropoiesis and expanded abnormal erythropoiesis, the so-called ***iron-loading anemias*** have low hepcidin levels and iron overload.

High-hepcidin anemias

- ***Hereditary***

Iron refractory iron deficiency anemia (IRIDA)

Hepcidin-producing adenomas

- ***Acquired***

Anemia of acute inflammation Common

Anemia of chronic inflammation (anemia of chronic disease)

Low-hepcidin anemias

- ***Hereditary – iron loading anemias***

β-thalassemia
Congenital dyserythropoietic anemia
Sideroblastic anemias

- ***Acquired***

Low risk MDS with ringed sideroblasts

Iron Refractory Iron Deficiency Anemia

- **IRIDA** is a rare recessive disorder characterized by **hypochromic microcytic anemia, low transferrin saturation, and inappropriately normal/high hepcidin levels.**

- It is caused by mutations of TMPRSS6, a gene that encodes matriptase-2.

- TMPRSS6, normally is highly expressed in the liver, inhibits hepcidin transcription by cleaving hemojuvelin.

- TMPRSS6 function is essential in iron ABSORPTION to allow the compensatory mechanism of increased iron absorption.

Iron Refractory Iron Deficiency Anemia

- **Matriptase-2 cleaves Hemojuvelin, a major regulator of hepcidin expression and this function is altered in this genetic form of anemia.**

- **In contrast to the low hepcidin levels observed in acquired iron deficiency, in patients with Matriptase-2 deficiency, serum hepcidin is inappropriately high for the low iron status and accounts for the absent/delayed response to oral iron treatment.**

- **A challenge for the clinicians and pediatricians is the recognition of the disorder among iron deficiency and other microcytic anemias commonly found in pediatric patients.**

- **The current treatment of IRIDA is based on parenteral iron administration**

Iron Refractory Iron Deficiency Anemia

- IRIDA is present since birth and usually diagnosed in childhood.
- Compared with classic iron deficiency, iron parameters in IRIDA are atypical and raise the suspicion of the disease.
- The percent saturation of transferrin is strongly reduced (less than 10%) as in other forms of iron deficiency
- **In IRIDA, in contrast to iron deficiency, levels of serum ferritin are normal/increased**. This reflects an increased ferritin accumulation in macrophages, due to the high hepcidin levels that induce store iron sequestration.
- **High Hepcidin/TSAT ratio is very sensitive**

(Camaschella, 2013; De Falco et al., 2013)

P. Bhatia et al. / Pediatric Hematology Oncology Journal 2 (2017) 48e53

How is iron-refractory iron deficiency anemia diagnosed?

- Lifelong anemia (hemoglobin 6-9 g/dL)
- very low MCV of 45-65 fL
- very low iron levels (transferrin saturation <5%)
- Abnormal oral iron absorption : no response to oral iron supplements or failure of an “oral iron challenge”
- Abnormal iron utilization: slow, incomplete, and transient response to parenteral iron (iron injected intravenously)
- Other affected family members with an autosomal recessive inheritance pattern

What is the oral iron challenge?

- The oral iron challenge is an easy test.

- First, your child will have a blood test to check anemia and iron levels. This will be followed by a dose of oral iron. About 90 minutes later, your child will receive a second blood test to check iron levels again.

- The iron level in the blood should rise significantly. If the iron level does not rise, it suggests either a problem with the small bowel or IRIDA.

How is IRIDA treated?

- The iron deficiency of IRIDA is refractory to oral iron supplementation and usually is only partially responsive to parenteral iron

- These repeated iron infusions can improve the anemia, microcytosis and iron stores (ferritin).

- However, the serum iron and transferrin saturation generally do not improve to the normal range, and if your child stops receiving regular intravenous iron infusions, the previous low iron levels and microcytic anemia almost certainly will appear again.

- **Manipulation of the hepcidin pathway with the aim of suppressing it might become an alternative therapeutic approach**

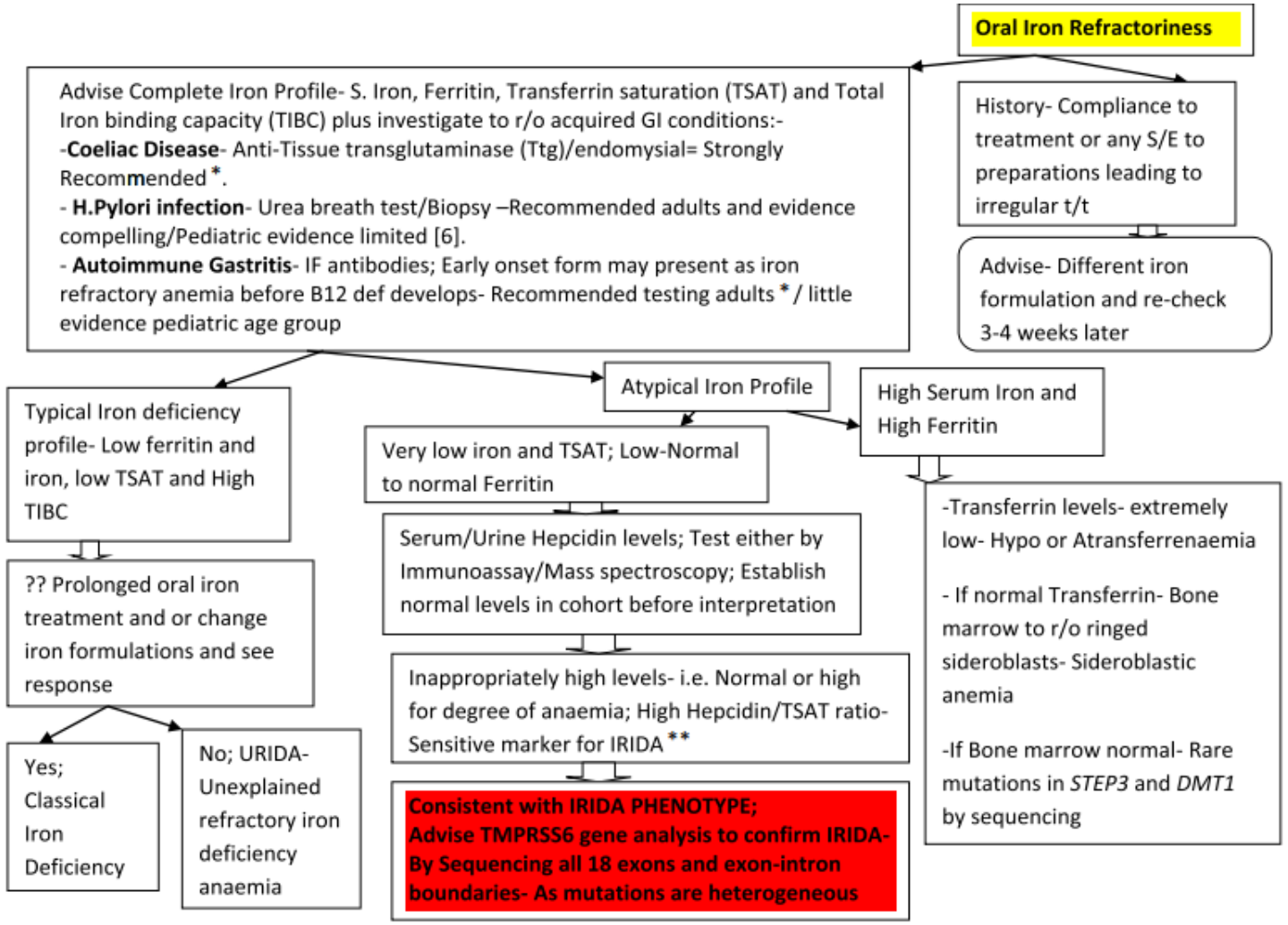

Fig. 1. Flow chart to highlight "SAID" (Structural Approach to IRIDA Diagnosis).
* Hershko and Skikne [24].
** Donker et al. [19].

Iron Refractory Iron Deficiency Anemia

- None of the tests proposed for IRIDA diagnosis covers 100% of the cases.
- The genetic test identifies that TMPRSS6 mutations, However, these tests are scarcely available.
- Serum hepcidin levels are usually increased/normal, independently of iron deficiency, and consistent with high/normal ferritin.
- Some patients with a phenotype of refractory iron deficiency have been reported to have a single TMPRSS6 mutated allele; here, the debate is whether they should be considered IRIDA or not.
- A spectrum of conditions exist ranging from classic severe IRIDA due to homozygous TMPRSS6 mutations to iron deficiency conferred by single mutations/polymorphic change

It is important to exclude inflammation

Salient features of review on IRIDA

- A structured approach is necessary in a patient appearing as iron deficient presents with oral iron refractoriness.
- This should include thorough history taking to rule out compliance issues and a testing for celiac disease and H. pylori infection.
- Hepcidin/TSAT ratio if high is a sensitive marker to identify cases of IRIDA phenotype and these cases should be likely subjected to TMPRSS6 gene analysis.
- Treatment in confirmed cases with IRIDA should be initiated with oral iron and vitamin C for 6-8 weeks as guidelines.
- Cases not responding or very minimally responding to above regimen should be planned for IV iron treatment.
- Siblings should be screened with a complete haemogram for presence of anemia and if present should be offered targeted mutation sequencing for a genetic diagnosis

- Hepcidin antagonists may be useful in inflammation and IRIDA.

- Hepcidin agonists may improve ineffective erythropoiesis. Correcting ineffective erythropoiesis ameliorates not only anemia but also iron homeostasis by reducing hepcidin inhibition.

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